IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Bror Morein et al.
Appln. No. : 10/550,026
Filed : June 11, 2007
Title : COMPOSITION COMPRISING ISCOM PARTICLES AND LIVE MICRO-ORGANISMS

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Examiner : Zachariah Lucas

Customer no. : 00116
Docket No.: : ALBI-41848

DECLARATION UNDER 37 CFR 1.132

Sir:

This Declaration under 37 CFR 1.132 is filed in response to the outstanding Office action of December 17, 2008.

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DECLARATION OF JAN FOHLMAN

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Jan Fohlman, having knowledge of the facts set forth herein, declares as follows:

1. Exhibits A, B, C, D, E, F, G, H, I, and J are attached and are made part of this declaration.

2. I presently reside at Research and Development Centre and Dept. of Infectious Diseases, Central Hospital, Växjö, Sweden.

3. I am an independent expert with regard to the subject matter of this application.

4. Regarding my independence, I am not a co-inventor of the subject matter claimed in the above-captioned patent application. I also do not have any legal or commercial claims or interests in the present patent application.

5. Regarding my expertise, I am an expert in the fields of microbiology and infectious disease, and I have conducted research involving ISCOMs.

6. My qualifications include the following. I earned a

Ph.D. in Biochemistry at the Institute of Biochemistry, Uppsala University, in 1977. I earned an M.D., also at Uppsala University, in 1986. I have been an Assistant Professor in Cell Research and in Infectious Diseases. I currently hold a combination position as Senior Consultant Infectious Diseases and Assistant Professor, R&D-Centre, Kronoberg County Council. Additional details of my qualifications are provided in Exhibit A.

7. I have authored or co-authored approximately 100 scientific papers that have been published in recognized journals and books. My publications include two published journal articles relating to research involving ISCOMs that I conducted in collaboration with Dr. Morein in the late 1980s and early 1990s. A list of my publications is provided as Exhibit B.

8. Adjuvants are used in killed vaccines to enhance immunogenicity of the vaccine antigens. Killed vaccines, particularly subunit vaccines, are far less immunogenic and require the addition of an adjuvant to reach acceptable immunity compared to, for example, live attenuated vaccines that replicate in a controlled manner to give a subclinical infection stimulating long-lived immunity.

9. The ISCOMs technology is an adjuvant technology developed and designed for subunit antigen preparations. The ISCOMs technology was developed during the 1980's as a potent means of conferring adjuvant activity to selected purified antigens obtained from extraction of microorganisms or as recombinant-DNA products. This is shown, for example, in Exhibit C, which corresponds to a scientific review article, Barr, I.G., and Mitchell, G.F., ISCOMs (Immunostimulating Complexes): The First Decade, Immunology & Cell Biology (1996), 74, 8-25. Specifically, Exhibit C, page 8, indicates

that "ISCOMs are . . . submicron, non-living particles composed of saponin, cholesterol, phospholipid, and in many cases protein".

10. I am aware that interpretation of terminology regarding incorporation of live microorganisms into ISCOMs is at issue in the prosecution of the present application. Specifically, U.S. Pat. No. 6,177,081, issued to Wechter et al., includes the following sentence: "Live attenuated viruses can also be incorporated into immunostimulating complexes (ISCOM) for use as a vaccine using methods well known in the art." Col. 9, lines 29-32. The statement has been cited by the Examiner as prior art to support the proposition that the Wechter reference teaches the combination of live attenuated viruses with an ISCOM. Office action dated July 28, 2009. The issue is whether the Wechter reference provides such a teaching. More specifically, the issue is whether the Wechter reference teaches that combination of a live attenuated virus per se with an ISCOM can be accomplished using methods well known in the art, rather than, for example, merely indicating the unremarkable, well established point that antigens derived based on solubilization, and thus killing, of a live attenuated virus can be incorporated into ISCOMs.

11. Based on my expertise as a scientist in the fields of microbiology and infectious diseases, including my extensive knowledge of vaccine technology and my experience conducting research relating to ISCOMs, and based on the evidence described below, it is my opinion that a person of ordinary skill in the art would understand the above-mentioned sentence from Wechter to mean that antigens derived based on solubilization, and thus killing, of a live attenuated virus can be incorporated into ISCOMs, not that live viruses per se can be incorporated. This is because, among numerous other

reasons to be discussed below, to the extent that similar terminology has been used in the ISCOMs field, the terminology has been used in reference to solubilization and thus killing of a microorganism prior to incorporation, not in reference to incorporation of the whole microorganism per se. This can be seen, for example, in Exhibit C, page 14, which states the following: "One potential problem in the preparation of ISCOMs is the incorporation of unwanted molecules when whole viruses are used. For example, when cell culture-derived HIV-1 virus was purified, solubilized and incorporated into ISCOMs, the major constituent was found to be HLA-DR which was derived from the host cell and forms part of the viral envelope." (Emphasis added). As can be seen, the quoted passage refers to methods for preparing ISCOMs based on use of "whole viruses" including an incorporation step, and thus uses terminology of incorporation of whole viruses into ISCOM particles. The passage elaborates on the meaning of this terminology, specifically by indicating that the methods refer to sequential steps of purification of whole virus, solubilization of the virus, and only then incorporation of the now solubilized remnants of the virus. As will be explained more fully below, solubilization of a virus (or other microorganism), in the context of incorporation into an ISCOM, relates to a process that destroys the structural integrity of the virus, resulting in killing of the virus. Thus, this passage demonstrates that the terminology of incorporation of whole viruses into ISCOMs refers not to incorporation of whole viruses per se but rather solubilization, and thus killing of the viruses, followed only then by incorporation into ISCOM particles. Accordingly, a person of ordinary skill in the art would understand also to refer to solubilization and thus killing of the live virus, followed only then by incorporation.

12. Consistent with this interpretation, the scientific literature does not apparently contain even a single reference that purports to show how a live microorganism per se could possibly be incorporated into an ISCOM particle, whereas the scientific literature includes numerous articles, including review articles, of which Exhibit C is one, that describe methods for incorporating antigens derived from microorganisms into ISCOMS. This can be seen, for example, based on Exhibit D, corresponding to the printed results of a PubMed search, conducted on June 14, 2009, based on the search terms "incorporation and live and iscom." As is well known among scientists, PubMed is a service of the U.S. National Library of Medicine that includes over 18 million citations from MEDLINE and other life science journals for biomedical articles back to 1948 and is a standard resource that scientists use to search for and identify articles in their field based on word searches. As shown in Exhibit D, the search yielded 31 articles. OF NOTE, THE APPLICANT DOES NOT ADMIT THAT ANY OF THE ARTICLES IDENTIFIED IN THIS PUBMED SEARCH ARE PRIOR ART. As explained in detail below, none of the 31 articles discloses any method for incorporation of a live microorganism per se into an ISCOM particle. To the extent that any of the articles uses terminology of incorporation of a live microorganism, the terminology is used in relation to solubilization and thus killing of the microorganism, followed by incorporation of the solubilized remnants of the killed microorganism into ISCOM particles.

13. Considering the results in more detail, reference 1 relates to comparison of an ISCOM-adjuvanted HMPV fusion protein subunit vaccine versus a live-attenuated vaccine that does not include ISCOMs. Reference 2 relates to inactivated, or in other words killed, microbes of a strain that otherwise would normally be used for live vaccine, mixed with pre-

formed, or in other words, antigen-free, ISCOMs. Reference 3 relates to use of killed microbes of a strain normally used for live vaccine. Reference 4 relates to use of a live strain for priming, followed by use of ISCOMs for booster, the priming composition and the booster composition not being mixed. Reference 5 relates to an ISCOM-based killed-strain vaccine. Reference 6, like reference 4, relates to use of a live strain for priming and ISCOMs for booster, the priming composition and booster composition not being mixed. Reference 7 relates to comparison of three formulations, a live formulation, an ISCOM formulation including antigens but not live microorganisms, and a lysate mixed with Quil A. Reference 8 relates to use of a live strain for priming and use of an ISCOM/non-living-rotavirus-like-particle for booster, again the priming composition and the booster composition not being mixed. Reference 9 relates to use of ISCOMs with or without antigens but not including live microorganisms. Reference 10 relates to influenza-ISCOMs, which correspond to ISCOMs in which the remnants of solubilized influenza viruses have been incorporated. Reference 11 relates to comparison of a formulation including MOMP protein-ISCOMs versus a formulation including live microorganisms. Reference 12 relates to inactivated vaccines. Reference 13 relates to comparison of ISCOM-based vaccines lacking live microorganisms versus a live attenuated virus vaccine lacking ISCOMs. Reference 14 relates to extraction of protein from microorganisms, followed by incorporation of the extracted protein into ISCOMs. Reference 15 relates to comparison of a live virus versus an ISCOM formulation lacking live microorganisms. Reference 16 relates to incorporation of antigens, extracted from influenza virus, into ISCOMs. References 17, 20, 21, 23, and 27 relate to incorporation of extracted antigens into ISCOMs. Reference 18 relates to comparison of a live attenuated vaccine versus other

formulations. Reference 19 relates to comparison of a live vaccine versus a formulation based on incorporation of antigens, extracted from virus, into ISCOMs. Reference 22 relates to various vaccines, none of which includes an ISCOM particle and a live microorganism together. Reference 24 relates to ISCOMs prepared from inactivated, or in other words, killed, Newcastle disease virus. Reference 25 relates to incorporation of disintegrated viral components into ISCOMs. Reference 26 relates to comparison of a live virus versus a formulation including ISCOMs into which F glycoprotein of measles virus has been incorporated. References 28, 29, and 30 relates to incorporation of viral proteins in ISCOMs. Remaining reference 31 relates to comparison of live attenuated vaccine versus a formulation including ISCOMs including viral proteins.

14. Of note, a word search for the term "iscom" alone yielded 472 articles, indicating that the PubMed database includes hundreds of articles that relate to ISCOMs. This is shown in Exhibit E, corresponding to the printed results of the first several pages of the search, including the titles of the first 20 articles identified in this search. THE APPLICANT DOES NOT ADMIT THAT ANY OF THE ARTICLES IDENTIFIED IN THIS PUBMED SEARCH ARE PRIOR ART.

15. Of further note, a word search for the terms "incorporation and iscom" yielded 276 articles, implying that the PubMed database includes hundreds of articles that relate to incorporation of compounds and structures into ISCOMs. This is shown in Exhibit F, corresponding to the printed results of the first several pages of the search, including the titles of the first 20 articles identified in this search. THE APPLICANT DOES NOT ADMIT THAT ANY OF THE ARTICLES IDENTIFIED IN THIS PUBMED SEARCH ARE PRIOR ART.

16. Given the absence any articles describing incorporation of live microorganisms per se into ISCOMs in the results of the PubMed search of Exhibit D and the multitude of articles relating to ISCOMs and incorporation of compounds therein in the results of the PubMed searches of Exhibits E and F, it is not plausible that the above-mentioned sentence from Wechter could be understood to indicate that methods for incorporation of live microorganisms per se into ISCOM particles were well known in the art at the time of filing of the Wechter application in the 1990s, nor even for that matter today.

17. Further consistent with this interpretation, it is common in the ISCOMs field to use terminology that on its face would seem to refer to an ISCOM particle into which a virus per se had been incorporated but which in fact corresponds to an ISCOM particle into which antigens derived from solubilized viruses have been incorporated, e.g. "influenza-ISCOMs" or "measles virus ISCOMs." This can be seen, for example, in Exhibit G, corresponding to a publication by Sjölander, S. et al., Intranasal immunization with influenza-ISCOM induces strong mucosal as well as systemic antibody and cytotoxic T-lymphocyte responses, Vaccine (2001), 19 4072-4080, which refers to "influenza-ISCOMs" and indicates that they are prepared based on disruption of influenza virus prior to incorporation. Specifically, Exhibit G, page 4073, indicates that "[i]nfluenza-ISCOMs were prepared as previously described," namely that "to a solution of disrupted A/PR8/34 virus was added a mixture of Quillaia saponin fractions (ISCOPREP(R)703, ISCOTEC AB), cholesterol (Sigma, USA) and di-palmitoyl phosphatidyl choline (Avanti, USA) dissolved in the detergent MEGA-10 (Sigma)." (Emphasis added). This can also be seen, for example, in Exhibit H, corresponding to a publication by Stittelaar, K.J. et al., Longevity of

Neutralizing Antibody Levels in Macaques Vaccinated with Quil A-Adjuvanted Measles Vaccine Candidates, Vaccine (2002) 21, 155-157. Specifically, Exhibit H, page 8, refers to a "measles virus-iscom," abbreviated as "MV-iscom," and indicates the MV-ISCOMs were prepared based on solubilization of the virus prior to incorporation of antigens into the ISCOMs, not based on incorporation of whole or live virus. This can be seen based on the statement within the reference that "[t]he iscom-matrixes were prepared identically to the MV-iscoms with the omission of solubilized MV." Exhibit H, page 8 (emphasis added). Accordingly, given that it is common in the ISCOM field to use terminology that on its face would seem to refer to a virus per se incorporated into an ISCOM particle but that actually refers to antigens of solubilized viruses incorporated into ISCOM particles, again, a person of ordinary skill in the art would understand the above-mentioned sentence from Wechter to refer specifically to solubilization and thus killing of the live virus, followed only then by incorporation.

18. Further, based on my expertise as a scientist in the fields of microbiology and infectious diseases, including my extensive knowledge of vaccine technology and my experience conducting research relating to ISCOMs, and based on the evidence discussed below, I state here with certainty that it is technically impossible to incorporate a live microorganism per se into an ISCOM, for at least the following reasons.

19. First, it would be physically impossible to incorporate any structure as large as a microorganism into an ISCOM particle. ISCOM particles are small in size, being only about 40 nm in diameter, and have a relatively low internal volume. The small size of ISCOM particles can be seen, for example, in Exhibit C, page 9, which states that "[t]ypically ISCOMs and ISCOM matrix particles are hollow, spherical, cage-

like particles that have a heterogenous size distribution of around 40 nm in diameter." The relatively low internal volume of ISCOM particles can be seen, for example, in Exhibit C, page 12, which indicates that "ISCOMs because of their very small size and internal volume are unable to encase [non-amphipathic] proteins" in the manner of a liposome. The size range of microorganisms is shown, for example, in Exhibit I, corresponding to the webpage "Bacteria, Fungi and Viruses, Sizes and Significance," http://www.ionizers.org/Sizes-of-Bacteria.html, which indicates that most microorganisms, including most viruses and all bacteria and fungi, are larger than 40 nm in diameter in size, many much larger, and that even those microorganisms that are not, namely a few viruses, are still at least 20 nm in diameter (see for example Paramyxovirus 0.022 µm coccus diameter). It can be appreciated from these references that the vast majority of microorganisms, including many types of viruses, could not conceivably be incorporated into an ISCOM particle because the microorganisms are larger than an ISCOM particle. It can also be appreciated from these references that even those few types of microorganisms that are smaller than an ISCOM particle, namely certain types of viruses, are not sufficiently small to be able to be incorporated into an ISCOM particle.
Specifically, even the smallest viruses would be too large to be encased within, or otherwise incorporated into, an ISCOM particle. This can be appreciated from the above-noted reference to Exhibit C, page 12, which indicates that non-amphipathic proteins cannot be encased within an ISCOM particle due to the small internal volume of the ISCOM particle. Viruses, including the smallest types, are well known to comprise pluralities of protein molecules, among other compounds, and thus are larger than individual protein molecules generally. Given that non-amphipathic proteins cannot be encased within an ISCOM particle due to the small

internal volume of the ISCOM particle, it follows that viruses also cannot be encased within an ISCOM particle also due to size. Thus, for at least these reasons, it would be physically impossible to incorporate any structure as large as a microorganism into an ISCOM particle.

20. Second, the methods that are actually well known in the art for incorporation of compounds or structures into ISCOMs are incompatible with maintenance of the structural integrity of, let alone survival of, any microorganisms. This is shown, for example, in Exhibit C, pages 11-13, which provides a broad review of methods known in the art for incorporating cell-derived molecules and other compounds and structures into ISCOM particles. Note specifically that Exhibit C indicates that "[w]hen ISCOMs include antigens derived from purified organisms or cell membranes, the molecules that are incorporated into ISCOMs are proteins or glycoproteins" and that "[t]hese molecules are extracted by detergent treatment and are integrated into the lipid-Quillaja saponin matrix when the detergent is removed and the ISCOMs are formed." Exhibit C, page 11. This passage describes the standard approach that is used for incorporation of antigens of microorganisms into ISCOMs, namely solubilization of microorganisms for purposes of extraction of proteins and other antigenic molecules and structures therein, followed by incorporation of those proteins, etc. into ISCOMs. The reference goes on to discuss numerous variations on this approach, none of which involve incorporation of live (or even whole) microorganisms per se into ISCOMs. To be clear, not every solubilization method would necessarily work with every microorganism, but every method for incorporation of antigens from any microorganism involve a step of effectively solubilizing the particular microorganism. Thus, for at least these reasons, the methods known in the art for incorporation

of any compounds or structures into ISCOMs would necessarily result in killing of the live microorganisms prior to any incorporation step.

21. I am further aware that the Wechter patent cites a publication of Fohlman within the paragraph including the above-mentioned sentence regarding incorporation of live virus into ISCOMs. Specifically, immediately following the above-mentioned sentence, the Wechter reference includes the following: "Activating Virus immunostimulating complexes (ISCOM)-vaccine containing viral capsid proteins to raise (sic) high neutralization antibody titres after two booster doses in Balb/c CUM and A/J inbred mouse strains. Fohlman, et al., supra." The citation apparently refers to work published by me in 1990. It is my opinion that the citation refers to my work because a search of the PubMed database using the terms "Fohlman and iscom" revealed only articles for which I am a co-author and because I am unaware of any other researcher named Fohlman in the ISCOM field. It is further my opinion that the citation refers specifically to my publication, included herein as Exhibit J, corresponding to Fohlman, J. et al., Vaccination of Balb/C Mice Against Enteroviral Mediated Myocarditis, 8 Vaccine 381 (1990). An analysis of the subject matter of this publication follows.

22. The publication, as provided in Exhibit J, does <u>not</u> disclose incorporation of a live microorganism per se into an ISCOM particle, but rather discloses disintegration of a killed virus followed by incorporation of the remnants of the killed virus into ISCOMs. More specifically, an ISCOM vaccine preparation was prepared with a Coxackie B3 virus. Coxackie B3 attenuated strain was used for the preparation. This particular virus preparation was killed by β-propiolactone (BPL) prior to introduction into the laboratory to protect personnel from hazard, and thus killed virus, not live virus,

was used in the preparation. To allow incorporation into ISCOMs, the virus particles must be disintegrated into sufficiently small sub-viral structures to reveal amphipathic surfaces that can interact with the lipids and saponin to form ISCOMs. The classical, most used, method for virus disintegration, detergent extraction, was not employed on the coxackie virus since the virus capsids are very stable and do not efficiently disintegrate by detergent treatment. Instead, in order to efficiently disrupt the virus into subunits the virus preparation was suspended in a 0.1 M Tris NaCl buffer adjusted to pH 9. UREA was added to 6M, EDTA to 0,002 M and DTT (dithiothreitol) to 0,002 M. Lipids (cholesterol and phosphatidyl choline) were added and Quil A (saponin) to a concentration of 0.1%. The mixture was incubated for 50 minutes at 37°C to ensure complete viral disruption and for further 50 minutes at room temperature for association of the viral subunits with the lipids and saponin. Successful ISCOM formation was verified by electron microscopy after extensive dialysis against PBS. The preparation was cleared from precipitate (aggregated material not incorporated into ISCOMs and nucleic acid) by centrifugation prior to further purification by pelleting through sucrose. The purified ISCOMs were analyzed by SDS-PAGE and were shown to contain viral proteins corresponding to VP1, VP2 and VP3, protein molecules with molecular weights of 24, 27 and 30 kD respectively.

23. As indicated above, this particular virus preparation was killed by β-propiolactone (BPL) prior to introduction into the laboratory to protect personnel from hazard. However, even if live virus had been used in the method, after the UREA-EDTA-DTT treatment no intact, let alone live, virus particles would be expected to be found in the preparation. If, for the sake of argument, intact virus particles were

present during the ISCOM preparation the intact virus particles would not incorporate since (1) the intact virus particle do not expose the amphipathic surfaces necessary for binding to lipids and saponin and (2) the intact virus particles are far too big, about 30 nm, to allow incorporation into a 40 nm ISCOM particle, for at least the reasons indicated above.

24. Any microorganism, virus bacteria or parasite would be killed after disintegration into sub-units that can incorporate into ISCOMs regardless of which method was used for disintegration or extraction. The methods employed for virus disintegration/extraction prior to ISCOM formation are in fact very similar to those used to prepare split-vaccine preparations.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the present application or any patent issued thereon.

Declarant Name: Jan Fohlman

Signature: _____

Country of Citizenship: SWEDEN

Address: RPD - Centre and Dept of Infectious Diseases Central Hospital SE-351 85 VÄXJÖ

Date: 15 June 2009

+46708 10 64 86